REMARKS

Claims 33-50 are pending and have been rejected. Claims 30, 32, 35, 36, and 49 have been cancelled. Claims 31, 33, 34, 37-48 and 50 remain in the case.

Applicants note with appreciation that the examiner has agreed that the claims of Group II and Group III should be examined together in this application.

Applicants provide herewith a proposed drawing correction to correct an error noted on Figure 3.

Applicants provide herewith a sequence listing to comply with the requirements of 37 CFR 1.821 through 1.825.

Claims 32, 33 and 34 are objected to under 37 CFR 1.75(l) for combining two active verbs in one paragraph. Claim 32 has been cancelled and claims 33 and 34 have been amended to recite separate steps.

Claims 35 and 36 are objected to under 37 CFR 1.75(c) as being of improper dependent form for failing to further limit the subject matter of a previous claim. Claims 35 and 36 have been cancelled.

Claims 30-50 are rejected under the second paragraph of Section 112 as being indefinite. The claims have been amended as suggested to address the concerns raised in the Action. Reconsideration and withdrawal of the rejection under the second paragraph of Section 112 is respectfully requested.

Claims 39-42 are rejected under the first paragraph of Section 112 as containing new matter. The examiner requests that applicants identify support in the specification regarding which component of the targetable conjugate is bound by the other arm of the bispecific antibody. The examiner's attention is directed to the paragraph bridging page 10 and 11 of the specification, which discloses that

> The targetable conjugate comprises a carrier portion which comprises or bears at least one epitope recognized by at least one arm of the bi-specific antibody or antibody fragment. In a preferred embodiment, the epitope is a hapten. In an alternative embodiment, the epitope is a part of the carrier. Examples of recognizable haptens include, but are not limited to, chelators, such as DTPA,

fluorescein isothiocyanate, vitamin B-12 and other moieties to which specific antibodies can be raised.

The specification clearly supports the concept that it is the epitope that is "recognized by" or binds to the other arm, and the wording of claims 39-42 has been changed to emphasize this. Reconsideration and withdrawal of the rejection under the first paragraph of Section 112 is respectfully requested.

Claims 33-34 are rejected under Section 101 as claiming the same invention as claims 35-37 and 43-44 of copending application No. 09/337,756. Inasmuch as this rejection is provisional, action will be deferred until one of the cases is allowed.

Claims 35-50 are rejected under the judicially-created doctrine of obviousness-type double patenting based on copending application No. 09/337,756. Again, since this rejection is provisional, action will be deferred until one of the cases is allowed.

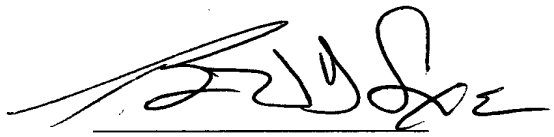
Claims 30-32, 35, 36 and 41 are rejected under Section 103(a) based on Barbet *et al.* (EP 0 263 046) in view of Bosslet *et al.* (U.S. 5,591,828). Claims 33-32, 35, 36 and 41 are rejected under Section 103(a) based on Barbet *et al.* (EP 0 263 046) in view of de Jonge *et al.* (*Mol. Imm.* 32:1405, 1995). Claims 30-32, 35, 36 and 41 are rejected under Section 103(a) based on Goodwin *et al.* (U.S. 4,803,713) in view of Bosslet *et al.* or de Jonge *et al* Claims 30-32, 35-38, 41-43 and 46 are rejected under Section 103(a) based on Karacay *et al.* (*Proceedings Am. Assoc. for Cancer Research Annual Meeting,* May 1999) or Karacay *et al.* (*J. Nucl. Med.*, 40(5): suppl., p. 255, 1999) in view of Bosslet *et al.* or de Jonge *et al.* Claims 37 and 38 are rejected under Section 103(a) based on Barbet *et al.*, Goodwin *et al.*, Bardies *et al.*, or Bautherot *et al.* in view of Bosslet *et al.*, or di Jonge *et al.* in further view of Goldenberg (WO 96/04313). In each of the rejections, the examiner cites one or more primary references alleged to teach a bispecific antibody with specificities as presently claimed, but that is made by chemical conjugation. The examiner then relies on Bosslet or de Jonge as teaching the production of antibodies in a host cell. The claims as amended all recite the production of bispecific Fab'-scFV fusion proteins in mammalian cells, and thus distinguish over the rejections based on these combinations. For example, the bacterially-produced bispecific of de Jonge has a molecular weight of 55,000, the size of a Fab'. Fab' fragments have a very short blood residence time due to rapid filtration by the kidney. This

markedly limits the percent of bispecific antibody that binds to the tumor or other target, thus markedly reducing the amount of the bivalent peptide that can be targeted to the tumor. Fab'-scFV fusion proteins as presently claimed, on the other hand, have a molecular weight above about 75,000, which is above the size threshold of proteins filtered by the kidney. Accordingly, no *prima facie* case of obviousness exists.

Claims 30-33, 35, 36, 41-43 and 46 are rejected under Section 103(a) based on Bardies *et al.* (*J. Nucl. Med.*, 37:1853, 1996) or Gautherot *et al.* (*J. Nucl. Med.*, 39:1937, 1998). Each of Bardies *et al.* and Gautherot *et al.* teach the production of antibodies by chemical coupling. There is no suggestion of antibodies produced by the methods as recited in applicants' claims.

In view of the foregoing remarks, it is believed that all claims are in condition for allowance. Reconsideration of all rejections and a notice of allowance are respectfully requested. Should there be any questions regarding this application, the examiner is invited to contact the undersigned attorney at the phone number listed below.

Respectfully submitted,



December 3, 2001
Date

Bernhard D. Saxe
Attorney for Applicants
Registration No. 28,665

FOLEY & LARDNER
3000 K Street, N.W., Suite 500
Washington, D.C. 20007-5109
Telephone: (202) 672-5300
Facsimile: (202) 672-5399

Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

Version with markings to show changes made in the claims

31. (Amended) A set of expression cassettes capable of producing a bi-specific [antibody or antibody fragment] Fab'-scFV fusion protein having at least one arm that specifically binds to a targeted tissue and at least one other arm that specifically binds to a targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes, wherein each cassette comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in a mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding [fragment of said bi-specific antibody] scFv linked to a Fd fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein expression of said fragment is under the control of said regulatory regions, wherein said set of expression cassettes produces a bispecific Fab'-scFV fusion protein when expressed in mammalian host cells.

33. (Amended) A method of preparing a bi-specific Fab'-scFv fusion protein having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes, comprising:

(1) (A) introducing into a mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding a scFv linked to a Fd fragment, and a transcriptional and translational termination regulatory region

functional in said mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) co-introducing into said mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said mammalian host cell a light-chain antibody fragment which is complementary to said Fd fragment in (A) and which when associated with said Fd fragment forms a [Fab] Fab' fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein expression of said light-chain antibody fragment is under the control of said regulatory regions;

(C) growing said cell; and

(D) isolating said bi-specific Fab'-scFV fusion protein, or

(2) (A) introducing into a first mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said first mammalian host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said first mammalian host cell, a translational initiation regulatory region functional in said first mammalian host cell, a DNA sequence encoding a scFv linked to a Fd fragment, and a transcriptional and translational termination regulatory region functional in said first mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) introducing into a second mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said second mammalian host cell a light-chain antibody fragment which is complementary to said Fd fragment in (2)(A) and which when associated with said Fd fragment forms a [Fab]

Fab' fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said second mammalian host cell, a translational initiation regulatory region functional in said second host cell, a DNA sequence encoding a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said second mammalian host cell, wherein expression of said light-chain antibody fragment is under the control of said regulatory regions;

(C) growing said first and second mammalian host cells;

(D) optionally isolating said bi-specific fusion protein fragment and said light-chain antibody fragment; [and]

(E) combining said fragments to produce a Fab'-scFV bi-specific fusion protein; and

(F) isolating said bi-specific fusion protein.

34. (Amended) A method of preparing a bi-specific Fab'-scFV fusion protein having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes, comprising:

(1) (A) introducing into a mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said mammalian host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding a scFv linked to a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein expression of said

fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) co-introducing into said mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said mammalian host cell a Fd fragment which is complementary to said light-chain antibody fragment in (A) and which when associated with said light-chain antibody fragment forms a [Fab] Fab' fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said host cell, a DNA sequence encoding a Fd fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein said expression of Fd fragment is under the control of said regulatory regions;

(C) growing said cell; and

(D) isolating said bi-specific Fab'-scFV fusion protein, or

(2) (A) introducing into a first mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said first mammalian host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said first mammalian host cell, a translational initiation regulatory region functional in said first mammalian host cell, a DNA sequence encoding a scFv linked to a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said first mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) introducing into a second mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said second mammalian host cell a Fd fragment which is complementary to said light-chain antibody fragment in (2)(A) and which when associated with said light-chain antibody

fragment forms a [Fab] Fab' fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said second mammalian host cell, a translational initiation regulatory region functional in said second mammalian host cell, a DNA sequence encoding a Fd fragment, and a transcriptional and translational termination regulatory region functional in said second mammalian host cell, wherein expression of said Fd fragment is under the control of said regulatory regions;

(C) growing said first and second mammalian host cells;

(D) optionally isolating said bi-specific fusion protein fragment and said Fd fragment; and

(E) combining said fragments to produce a bi-specific Fab'-scFV fusion protein; and

(F) isolating said bi-specific fusion protein.

does not show as amended

37. The construct of claim [30] 33, wherein said at least one arm that specifically binds a targeted tissue is a humanized antibody or a fragment of a humanized antibody.

38. The construct of claim [30] 33, wherein said at least one other arm that specifically binds a targetable conjugate is a humanized antibody or a fragment of a humanized antibody.

39. (Amended) The construct of claim [30] 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a peptide.

40. (Amended) The construct of claim [30] 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a carbohydrate.

41. (Amended) The construct of claim [30] 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a hapten.

42. (Amended) The construct of claim [30] 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a chelator or a metal-chelate complex.

43. The construct of claim 42, wherein said chelator is a hard base chelator for a hard acid cation.

44. The construct of claim 42, wherein said chelator is a soft base chelator for a soft acid cation.

45. The construct of claim 43, wherein said chelator is a hard base chelator that comprises carboxylate and amine groups.

46. The construct of claim 43, wherein said hard base chelator is DTPA, NOTA, DOTA or TETA.

47. (Amended) The construct of claim [30] 33, wherein said at least one other arm specifically binds a tyrosyl-lysine dipeptide.

48. (Amended) The construct of claim [30] 33, wherein said at least one other arm specifically binds Tyr-Lys(DTPA)-NH_2, or Lys(DTPA)-Tyr-Lys(DTPA)-NH_2..

50. (Amended) The set of expression cassettes of claim [49] 31, wherein a second expression cassette is capable of producing in a mammalian host cell a light-chain antibody fragment which is complementary to said Fd fragment in and comprises, in the 5'

to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein expression of said light-chain antibody fragment is under the control of said regulatory regions, and wherein said light-chain antibody fragment, when associated with said Fd fragment, forms a [Fab] Fab' fragment whose binding site is specific for said targeted tissue.

Version with markings to show changes made in the specification

Please amend the paragraph bridging pages 12 and 13 as follows:

Carriers having as few as one amine residue may be used, preferably two to ten amino acid residues, if also coupled to other moieties such as chelating agents. Examples include modified amino acids, such as bis-DTPA-lysine, and bis-DTPA-diamine. These agents can be linked covalently to molecules which are to be targeted. The hapten moiety of the carrier portion should be a low molecular weight conjugate, preferably having a molecular weight of 100,000 daltons or less, and advantageously less than about 20,000 daltons, 10,000 daltons or 5,000 daltons, including the metal ions in the chelates. For instance, the known peptide di-indium-DTPA-Tyr-Lys(DTPA)-OH has been used to generate antibodies against the indium-DTPA portion of the molecule. However, by use of the non-indium-containing molecule, and appropriate screening steps, new Abs against the tyrosyl-lysine dipeptide can be made. More usually, the antigenic peptide will have four or more residues, such as the peptide Ac-Phe-Lys(DTPA)-Tyr-Lys(DTPA)-NH_2 (SEQ ID NO: 1). Again, the non-metal-containing peptide is used as an immunogen, with resultant Abs screened for reactivity against the Phe-Lys-Tyr-Lys (SEQ ID NO: 1) backbone.

Please amend the paragraph at lines 13-28 on page 14 as follows:

In another embodiment of the invention, the haptens of the targetable conjugate comprise a known immunogenic recognition moiety, for example, a known hapten. Using a known hapten, for example, fluorescein isothiocyanate (FITC), higher specificity of the targetable conjugate for the antibody is exhibited. This occurs because antibodies raised to the hapten are known and can be incorporated into the inventive antibody. Thus, binding of the targetable conjugate with the attached chelator or metal-chelate complex would be highly specific for the inventive antibody or antibody fragment. Another example of a hapten to be substituted onto the targetable conjugate includes vitamin B12. The use of vitamin B12 is advantageous since anti-B12 Mabs are known and no free serum B12 exists, therefore, great specificity for the antibody may be exhibited. An example of a targetable conjugate containing a hapten includes Ac-Cys-(S-Bz-DTPA)-Gly-Lys-(N-FITC)-Tyr-Cys-(S-Bz-DTPA)NH_2 (SEQ ID NO: 2). The chelator or its chelate with a metal cation also can

function as the hapten to which an antibody is raised. Another example of a hapten to be conjugated to a targetable conjugate includes biotin.

Please amend the paragraph bridging pages 24 and 25 as follows:

Chelators such as those disclosed in U.S. Patent 5,753,206, especially thiosemicarbazonylglyoxylcysteine(TscG-Cys) and thiosemicarbazinyl-acetylcysteine (TscA-Cys) chelators are advantageously used to bind soft acid cations of Tc, Re, Bi and other transition metals, lanthanides and actinides that are tightly bound to soft base ligands, especially sulfur- or phosphorus-containing ligands. It can be useful to link more than one type of chelator to a peptide, e.g., a DTPA or similar chelator for, say In(III) cations, and a thiol-containing chelator, e.g., TscG-Cys, for Tc cations. Because antibodies to a di-DTPA hapten are known (Barbet '395, *supra*) and are readily coupled to a targeting antibody to form a bsAb, it is possible to use a peptide hapten with non-radioactive diDTPA chelates and another chelate for binding a radioisotope, in a pretargeting protocol, for targeting the radioisotope. One example of such a peptide is Ac-Lys(DTPA)-TyrLys(DTPA)-Lys(TscG-Cys-)-NH_2 (SEQ ID NO: 3). This peptide can be preloaded with In(III) and then labeled with 99-m-Tc cations, the In(III) ions being preferentially chelated by the DTPA and the Tc cations binding preferentially to the thiol-containing TscG-CysC. Other hard acid chelators such as NOTA, DOTA, TETA and the like can be substituted for the DTPA groups, and Mabs specific to them can be produced using analogous techniques to those used to generate the anti-di-DTPA Mab.

Please amend the paragraph at lines 9-27 on page 27 as follows:

In another embodiment, the present invention can be used in Boron Neutron Capture Therapy (BNCT) protocols. BNCT is a binary system designed to deliver ionizing radiation to tumor cells by neutron irradiation of tumor-localized boron-10 atoms. BNCT is based on the nuclear reaction which occurs when a stable isotope, isotopically enriched B-10 (present in 19.8% natural abundance), is irradiated with thermal neutrons to produce an alpha particle and a Li-7 nucleus. These particles have a path length of about one cell diameter, resulting in high linear energy transfer. Just a few of the short-range 1.7 MeV

alpha particles produced in this nuclear reaction are sufficient to target the cell nucleus and destroy it. Success with BNCT of cancer requires methods for localizing a high concentration of boron-10 at tumor sites, while leaving non-target organs essentially boron-free. Compositions and methods for treating tumors in patients using pre-targeting bsAb for BNCT are described in [U.S.S.N. 09/205,243] U.S. Patent No. 6,228,362 and can easily be modified in accordance with the present invention. Additionally, other elements are suitable for neutron capture reactions. One example is uranium. Uranium, in large amounts, can be bound by naturally occurring chelating agents such as ferritin. Such strategies have been described in [U.S.S.N. **] U.S. Patent No. 6,228,362 , are easily adaptable to the present invention and are hereby incorporated in their entirety by reference.

Please amend the paragraph bridging pages 27 and 28 as follows:

In another embodiment of the practice of the invention, the bsAb is administered prior to administration of a diagnostic agent which is associated with the targetable conjugate. After sufficient time has passed for the bsAb to target to the diseased tissue, the diagnostic agent is administered. Subsequent to administration of the diagnostic agent, imaging can be performed. Tumors can be detected in body cavities by means of directly or indirectly viewing various structures to which light is delivered and then collected. Lesions at any body site can be viewed so long as nonionizing radiation can be delivered and recaptured from these structures. For example, positron emission tomography (PET) which is a high resolution, non-invasive, imaging technique can be used with the inventive antibodies for the visualization of human disease. In PET, 511 keV gamma photons produced during positron annihilation decay are detected. Similar pre-targeting strategies for PET using Fluorine-18 and Gallium-68 have been described, respectively in [U.S.S.N. 09/146,318 and (second serial number is pending)] U.S. patent No. 6,187,284 and U.S. Patent No. 6,071,490. The methodologies described in these applications are easily adaptable to the present invention and are hereby incorporated in their entirety by reference.

Please amend the paragraph at lines 1-19 on page 29 as follows:

Certain cytotoxic drugs that are useful for anticancer therapy are relatively insoluble in serum. Some are also quite toxic in an unconjugated form, and their toxicity is considerably reduced by conversion to prodrugs. Conversion of a poorly soluble drug to a more soluble conjugate, *e.g.*, a glucuronide, an ester of a hydrophilic acid or an amide of a hydrophilic amine, will improve its solubility in the aqueous phase of serum and its ability to pass through venous, arterial or capillary cell walls and to reach the interstitial fluid bathing the tumor. Cleavage of the prodrug deposits the less soluble drug at the target site. Many examples of such prodrug-to-drug conversions are disclosed in [Hansen U.S.S.N. 08/445,110] U.S. Patent No. 5,851,527.

Please amend the paragraph at lines 22-33 on page 32 as follows:

A variety of carriers are well-suited for conjugation to prodrugs, including polyamino acids, such as polylysine, polyglutamic (E) and aspartic acids (D), including D-amino acid analogs of the same, co-polymers, such as poly(Lys-Glu) {poly[KE]}, advantageously from 1:10 to 10:1. Copolymers based on amino acid mixtures such as poly(Lys-Ala-Glu-Tyr) (SEQ ID NO: 4) (KAEY; 5:6:2:1) can also be employed. Smaller polymeric carriers of defined molecular weight can be produced by solid-phase peptide synthesis techniques, readily producing polypeptides of from 2-50 residues in chain length. A second advantage of this type of reagent, other than precise structural definition, is the ability to place single or any desired number of chemical handles at certain points in the chain. These can be used later for attachment of recognition and therapeutic haptens at chosen levels of each moiety.

Please amend examples 1 and 2 on page 40 as follows:

Example 1) Synthesis of a Peptide Antigen:

The peptide, Ac-Phe-Lys(Ac)-Tyr-Lys(Ac)-OH (SEQ ID NO: 1), is assembled using a resin for solid-phase synthesis and attaching the first residue (lysine) to the resin as the differentially protected derivative alpha-Fmoc-Lys(Aloc)-OH. The alpha-

Fmoc protecting group is selectively removed and the Fmoc-Tyr(OBut), alpha-Fmoc-Lys(Aloc)-OH, and Fmoc-Phe-OH added with alternate cycles of coupling and alpha-amino group deprotection. The Aloc - and OBut- side-chain protecting groups are then removed by reaction with TFA and the free alpha- and epsilon-amino groups are capped by reaction with acetic anhydride to give Ac-Phe-Lys(Ac)-Tyr-Lys(Ac)-OH (SEQ ID NO: 1).

Example 2) Coupling of Ac-Phe-Lys(Ac)-Tyr-Lys(Ac)-OH (SEQ ID NO: 1) to KLH:

The peptide, Ac-Phe-Lys(Ac)-Tyr-Lys(Ac)-OH (SEQ ID NO: 1), dissolved in water and pH-adjusted to 4.0 with 1N HC1, is treated with a molar equivalent of 1-ethyl-3(3-dimethylaminopropyl) carbodiimide and allowed to react for 1 h at 4°C. Keyhold limpet hemocyanin (KLH) buffered at pH 8.5 is treated with a 100-fold molar excess of the activated peptide and the conjugation reaction is allowed to proceed for 1 h at 4°C. The peptide-KLH conjugate is purified from unreacted peptide by size-exclusion chromatography and used for antibody production.

Please amend Examples 9, 10 and 11 on pages 42 and 43 as follows:

Example 9) Synthesis of Ac-Phe-Lys(Bz-DTPA)-Tyr-Lys(Bz-DTPA)-NH_2 (SEQ ID NO: 1):

The peptide, Ac-Phe-Lys(DTPA)-Tyr-Lys(DTPA)- NH_2 (SEQ ID NO: 1), is assembled using a resin for solid-phase synthesis and attaching the first residue (lysine to said resin as the differentially protected derivative alpha-Fmoc-Lys(Aloc)-OH. The alpha-Fmoc protecting group is selectively removed and the Fmoc-Tyr(OBut), alpha-Fmoc-Lys(Aloc)-OH, and Fmoc-Phe-OH added with alternate cycles of coupling and alpha-amino group deprotection. The Aloc- side-chain is removed by reaction with palladium (0) catalyst. Alternatively, Boc-group protecting groups may be used which may be removed by reaction with TFA and the free amino groups reacted with excess of the ITC-Bz-DTPA. After removing excess Bz-DTPA, the alpha-amino group is capped by reaction with acetic anhydride, and the entire peptide removed from the resin with TFA (with concomitant

deprotection of the tyrosyl residue) to give Ac-Phe-Lys(Bz-DTPA)-Tyr-Lys(Bz-DTPA)-NH_2 (SEQ ID NO: 1).

Example 10) Radiolabeling of Ac-Phe-Lys(Bz-DTPA)-Tyr-Lys(Bz-DTPA)-NH_2 (SEQ ID NO: 1) with Y-90:

The title peptide in 100-fold molar excess is mixed with yttrium-90 radionuclide in acetate buffer at pH 5.5. The radiolabeling is complete and quantitative after 30 minutes.

Example 11) Conjugation of a Carboxylesterase to di-DTPA-Peptide:

Carboxylesterase (5 mg) in 0.2 M phosphate buffer, pH 8.0, is treated with a five-fold molar excess of the cross-linking agent sulfo-succinimidyl-[4-maleimidomethyl]-cyclohexane-1-carboxylate (sulfo-SMCC). After stirring two hours at room temperature, the activated enzyme is separated from low molecular weight contaminants using a spin-column of G-25 Sephadex and equilibrated in 0.1 M phosphate buffer, pH 7, containing 1 mM EDTA. The tetrapeptide N-acetyl-Cys.Lys(DTPA).Tyr.Lys(DTPA).NH_2 (SEQ ID NO: 5) (ten-fold molar excess) is added to the activated enzyme and dissolved in the same buffer as used in the spin-column. After stirring for one hour at room temperature, the carboxylesterase-Cys.Lys(DTPA).Tyr.Lys(DTPA).NH_2 (SEQ ID NO: 5) peptide conjugate is purified from unreacted peptide by spin-column chromatography on G-25 Sephadex in 0.25 M acetate buffer, pH 6.0. Successful conjugation is demonstrated by indium-111 labeling of an aliquot of the conjugate, and analysis by size-exclusion HPLC.

Please amend Example 15 on pages 44 and 45 as follows:

Example 15) Synthesis of Ac-Lys(DTPA)-TyrLys(DTPA)-Lys(TscG-Cys-)-NH_2 (SEQ ID NO: 3) (IMP 192):

The first amino acid, Aloc-Lys(Fmoc)-OH was attached to 0.2 1 mmol Rink amide resin on the peptide synthesizer followed by the addition of the Tc-99m ligand binding residues Fmoc-Cys(Trt)-OH and TscG to the side chain of the lysine using standard Fmoc automated synthesis protocols to form the following peptide: Aloc-Lys(TscG-Cys(Trt)-rink

resin. The Aloc group was then removed by treatment of the resin with 8 mL of a solution containing 100 mg $Pd[P(Ph)_3]_4$ dissolved in 10 mL CH_2Cl_2, 0.75 mL glacial acetic acid and 2.5 ml diisopropylethyl amine. The resin mixture was then treated with 0.8 ml tributyltin hydride and vortex mixed for 60 min. The peptide synthesis was then continued on the synthesizer to make the following peptide: Lys(Aloc)-Tyr-Lys(Aloc)-Lys(TscG-Cys-)(SEQ ID NO: 3)-rink resin. The N-terminus was acetylated by vortex mixing the resin for 60 mm with 8 mL of a solution containing 10 mL DMF, 3 mL acetic anhydride, and 6 mL diisopropylethylamine. The side chain Aloc protecting groups were then removed as described above and the resin treated with piperidine using the standard Fmoc deprotection protocol to remove any acetic acid which may have remained on the resin. Activated DTPA and DTPA Addition: The DTPA, 5 g was dissolved in 40 mL 1.0 M tetrabutylammonium hydroxide in methanol. The methanol was removed under hi-vacuum to obtain a viscous oil. The oil was dissolved in 50 mL DMF and the volatile solvents were removed under hi-vacuum on the rotary evaporator. The DMF treatment was repeated two more times. The viscous oil was then dissolved in 50 ml DMF and mixed with 5 g HBTU. An 8 ml aliquot of the activated DTPA solution was then added to the resin which was vortex mixed for 14 hr. The DTPA treatment was repeated until the resin gave a negative test for amines using the Kaiser test. Cleavage and Purification: The peptide was then cleaved from the resin by treatment with 8 ml of a solution made from 30 ml TFA, 1 ml triisopropylsilane, and 1 ml ethanedithiol for 60 mm. The crude cleaved peptide was precipitated by pouring into 30 ml ether and was collected by centrifugation. The peptide was then purified by reverse phase HPLC using a 4 x 30 cm Waters preparative C-18 Delta-Pak column (15 μm, 100Å). The HPLC fractions were collected and lyophilized to obtain a fraction which contained the desired product by ESMS ($MH \pm 1590$). Kit Formulation: The peptide was formulated into lyophilized kits which contained 78 μg of the peptide, 0.92 mg non-radioactive $InCl_3$, 100 μg stannous chloride, 3 mg gentisic acid, and HPCD (10 % on reconstitution).

Please amend example 21 on pages 48-51 as follows:

Example 21) Construction and expression of hMN14Fab-734scFv:

Recombinant methods were used to produce a monovalent bi-specific fusion protein comprising a Fab fragment derived from a humanized monoclonal anti-CEA antibody and a scFv derived from a murine anti-diDTPA. See Figure 3. The structure of single chain 734 (734scFv) was designed as GGGS-VL (SEQ ID NO: 6)-(GGGGS)3-VH (SEQ ID NO: 7), in which the proximal GGGS (SEQ ID NO: 6) provides a flexible linkage for the scFv to be connected to the constant region of the heavy chain of hMN-14 (Figure 1). Alternatively, the scFv can be connected to the constant region of the light chain of hMN-14. Appropriate linker sequences necessary for the in-frame connection of the hMN14 heavy chain Fd to 734scFv were introduced into the VL and VK domains of 734 by PCR reactions using specific primer sets. PCR-amplification of 734VL was performed using the primer set 734VLscFv5'(Cys) and 734VLscFv3' (respectively, SEQ ID NO's: 1 & 2). The primer 734VLscFv5'(Cys) represents the sense-strand sequence encoding the first four residues (PKSC (SEQ ID NO: 8)) of the human IgG1 hinge, linked in-frame to the first six residues (QLVVTQ (SEQ ID NO: 9)) of 734 VL, via a short flexible linker, GGGS (SEQ ID NO: 6). One cysteine of the human hinge was included because it is required for the interchain disulfide linkage between the hMN14 heavy chain Fd-734scFv fusion and the hMN14 light chain. A Pst1 site was incorporated (underline) to facilitate ligation at the intronic sequence connecting the CH1 domain and the hinge. The primer 734VLscFv3' represents the anti-sense sequence encoding the last six residues (TKLKIL (SEQ ID NO: 10)) of the 734 VL domain and a portion of the flexible linker sequence (GGGGSGGGG (SEQ ID NO: 11)), which is fused in-frame downstream of the VL domain. Following PCR amplification, the amplified product (~400 bp) first was treated with T4 DNA polymerase to remove the extra "A" residue added to the termini during PCR-amplification and subsequently was digested with Pst1. The resultant product was a double-stranded DNA fragment with a Pst1 overhang and a blunt end. PCR amplification of 734VH was performed using the primer set 734VHscFv5' and 734VHscFV3'(Sac1). Primer 734VHscFv5' (SEQ ID NO: 3) represents the sense-strand sequence encoding the remaining part of the flexible linker sequence (SGGGGS(SEQ ID NO: 12)) connecting the VL and VH sequences, and the first six residues (EVKLQE (SEQ ID NO: 13)) of the 734 VH domain. Primer 734VHscFv3'(Sac1) (SEQ ID NO: 4) represents the anti-sense sequence encoding the last six residues (TVTVSS (SEQ ID NO: 14)) of 734 VH. Also included is a translation stop codon (*). The restriction sites Eag1 (bold) and

Sac1(underlined) were incorporated downstream of the stop codon to facilitate subcloning. Similarly, the PCR-amplified VH product of ~400 bp was first treated with T4 DNA polymerase to remove the extra "A" residues at the PCR product termini, and then digested with Sac1, resulting in a VH DNA fragment with a blunt end-sticky end configuration. A pBlueScript (Stratagene, La Jolla)-based staging vector (HC1kbpSK) containing a SacII fragment of the human IgG1 genomic sequence was constructed. The genomic SacII fragment contains a partial 5' intron, the human IgG1 CH1 domain, the intronic sequence connecting the CH1 to the hinge, the hinge sequence, the intronic sequence connecting the hinge to the CH2 domain, and part of the CH2 domain. The segment containing the hinge and part of the CH2 domain in HC1kbpSK was removed by Pst1/Sac1 digestion, and the cloning site generated was used to co-ligate the VL (Pst1/blunt) and VH (blunt/Sac1) PCR products prepared above. The CH1 domain in the resultant construct (CH1-734pSK) is connected to the 734scFv gene sequence via an intron (Figure 4). Since the genomic SacII fragment for IgG1 only included part of the 5' intron sequence flanking the CH1 domain, the full intronic sequence was restored by inserting the remaining intronic sequence as a BamH1/SacII segment, into the corresponding sites of the CH1-734pSK. The BamH1/Eag1 fragment containing the full 5' intron, CH1 domain, connecting intron, 5 hinge-residues, short GGGS linker, and a 734scFv sequences was then isolated, and used to replace the HindIII/Eag1 segment containing the human genomic IgG1 constant sequence in the hMN14pdHL2 vector. A HNB linker (SEQ ID NO: 5) with a BamH1 overhang on one end and a HindIII overhang on the other was used to facilitate the BamH1/Eag1 fragment ligation into the HindIII/Eag1 site in the hMN14pdHL2 vector. The resultant vector was designated hMN14-734pdHL2 and can be used to transfect mammalian cells for the expression of the bispecific protein. The hMN14pdHL2 vector was derived from the vector, pdHL2, which has previously been described. See Losman *et al.*, *Cancer Supplement*, 80:2660, 1997. Construction of hMN14pdHL2 was performed by replacing the VH and VK domains of hLL2pdHL2 with that of hMN14 using standard molecular biology techniques (Figure 5). The hMN14-734pdHL2 vector was transfected into SP2/0 cells by electroporation and the cell clones secreting bsAb were identified. The bsAb purified from cell culture supernatant (clone 341.1G6) on a protein L column (Pierce, Rockford, IL) is a 75 kD protein (based on amino acid sequence calculation) that co-migrated with the 66 kD marker in non-reducing SDS-PAGE probably due to secondary

structure (Figure 2, lane 2). Under reducing conditions, bands corresponding to a heavy (50 kD) and a light (25 kD) chain were observed (Figure 2, lane 4). Kappa chain monomers (25 kD) and dimers (50 kD) secreted by the transfectoma also were co-purified (Figure 2, lane 2) since protein L binds to kappa light chains of human, mouse and rat. Further separation of bsAb from kappa mono- and dimers is accomplished with ion-exchange chromatography. Purified hMN14Fab-734scFv shows specific binding to both CEA and In-DTPA-BSA in a dose dependent manner.

Please amend example 26 on page 56 as follows:

Example 26) Use of anti-CEA-IgG x anti-peptide Fab' Bi-specific Ab for Prodrug Therapy with a bsAb Clearing Step:

A patient with colorectal cancer is given an injection of an IgG-hMN-14 x anti-peptide Fab' bsAb. After 48 h, to allow for maximum accretion in tumors, the patient is given a clearing dose of galactose-WI2-Fab'. This amount is between 5 and 15 times the amount of primary bsAb remaining in circulation at the time-point specified. Three hours after administration of the galactose-W12-Fab', a tumor-saturating amount of the carboxylesterase-Cys.Lys(DTPA).Tyr.Lys(DTPA).NH_2 (SEQ ID NO: 5) conjugate from example 11 is given, and allowed to clear circulation and normal tissues. After an additional three hours, a standard chemotherapy dose of CPT- 11 is administered to the patient. This protocol effectively generates free SN-38 specifically at the tumor target sites and effects the destruction of tumor cells.

Please replace the paragraph before Table 11 on page 62 as follows:

The experiment was repeated with a lyophilized kit of IMP 225 (Ac-Cys(Dox-$COCH_2$-)-Lys(DTPA)-Tyr-Lys(DTPA)-NH_2 (SEQ ID NO: 5) MNa^+ 1938), containing 11 micrograms of peptide.